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Control of enantioselectivity of lipase-catalyzed esterification in supercritical carbon dioxide by tuning the pressure and temperature

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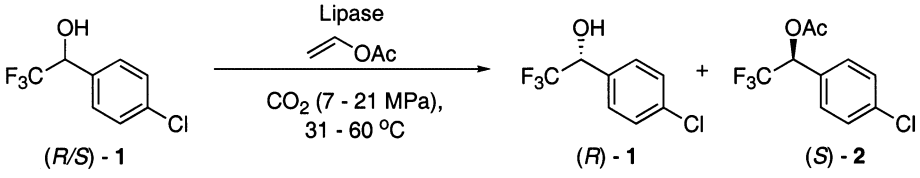
Abstract—The enantioselectivity of lipase-catalyzed esterification of 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol in supercritical carbon dioxide was controlled by tuning the pressure and temperature. The enantioselectivity was higher at low pressure and low temperature ($E=60$) than at high pressure and high temperature ($E=10$). At the density of 0.75 g/mL, a modified Eyring plot of $\ln E$ against $1/T$ was found to be linear, as expected from the theory on the effects of temperature on stereochemistry. © 2003 Published by Elsevier Science Ltd.

1. Introduction

Supercritical carbon dioxide ($scCO_2$, critical point: 31.0°C, 7.38 MPa (72.9 atm)) has recently attracted attention as an environmentally friendly solvent for extraction, chemical reactions and chromatography.[1] Moreover, its properties, such as density, dielectric constant, diffusivity, viscosity, solubility etc. can be tuned by adjusting the pressure and temperature, which clearly distinguishes this supercritical fluid from conventional solvents. Therefore, with supercritical fluids, solvent effects on the reaction can be examined without changing the kind of solvent, and continuous change in a reaction can be expected since the solvent properties can be changed continuously by manipulating the pressure and temperature.

Our aim is to control the biocatalytic reaction in $scCO_2$ by adjusting the pressure and temperature. Such attempts to control enzymatic reactions have been done by using supercritical fluoroform because its polarity changes drastically with pressure and temperature.[1,2] For example, Mori et al. demonstrated the reversible control of transglycosylation by a lipid-coated β-D-galactosidase[2a] and enantioselective esterification by a lipid-coated lipase.[2b] Kamat et al. examined the enantioselectivity of Subtilisin carlsberg and an Aspergillus protease[2c,d] in supercritical fluoroform. However, only a few reports on the control of biocatalytic reactions using $scCO_2$ under various pressures and temperatures have been reported.[3] We have examined the enantioselective acetylation of racemic 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol (*RS*)-**1** with lipases and vinyl acetate[4] in $scCO_2$ as shown in Scheme 1.[5]



Scheme 1.

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The fluorinated compound was chosen as a model substrate because enantiomerically pure fluorinated alcohols have received much attention for the synthesis of ferroelectric liquid crystals or bioactive compounds.[6] We found that the enantioselectivity of a reaction using the lipase Novozym can be controlled by adjusting the pressure and temperature of $scCO_2$. Moreover, at the density of 0.75 g/mL (31°C at 9.5 MPa, 35°C at 11.2 MPa, 40°C at 13.2 MPa, 45°C at 15.3 MPa, 50°C at 17.5 MPa, 55°C at 19.6 MPa, and 60°C at 21.8 MPa),[7] the modified Eyring plot of ln E[8] against $1/T$ was found to be linear, which correlates well with results predicted by the theory of the effects of temperature on enantiochemistry.[9]

2. Results and discussion

2.1. Screening of lipases

We screened various lipases for the enantioselective acetylation of (*RS*)-**1** with vinyl acetate[4] in $scCO_2$ at 9.1 and 14.5 MPa. Lipases tested were LPL (*Pseudomonas aeruginosa*), AY (*Candida rugosa*), AH (*Pseudomonas cepacia*), PS-D (*Pseudomonas cepacia*), PS-C (*Pseudomonas cepacia*), Lipozyme (*Rizomucor miehei*), and Novozym (*Candida antarctica*). The results are listed in Table 1. The (*S*)-enantiomer reacted faster than the (*R*)-enantiomer, affording the (*S*)-acetate (*S*)-**2** and the remaining (*R*)-alcohol (*R*)-**1** except when lipase AY was used at 9.1 MPa. The highest enantioselectivity[8] ($E=38$) was obtained using Novozym at 9.1 MPa. Interestingly, the enantioselectivity was significantly affected by the pressure.

Table 1. Screening of lipases for enantioselective acetylation of (*RS*)-**1** in supercritical carbon dioxide

Lipase	Low pressure conditions (9.1 MPa)		High pressure conditions (14.5 MPa)	
	Yield (%)	E[a]	Yield (%)	E[a]
LPL (*Pseudomonas aeruginosa*)	52	12	38	16
AY (*Candida rugosa*)	8	1[b]	2	2
AH (*Pseudomonas cepacia*)	3	29	0	–
PS-D (*Pseudomonas cepacia*)	0	–	0	–
PS-C (*Pseudomonas cepacia*)	43	8	22	17
Lipozyme (*Rizomucor miehei*)	0	–	0	–
Novozym (*Candida antarctica*)	25	38	24	23

Reaction conditions: 40°C, 4 h.
[a] The (*S*)-enantiomer reacted faster than (*R*)-enantiomer.
[b] In this case, the (*R*)-enantiomer reacted slightly faster than the (*S*)-enantiomer.

2.2. Time course of the reaction using Novozym

The enantioselective acetylation of (*RS*)-**1** with vinyl acetate in $scCO_2$ by Novozym was investigated at 55°C and 10 MPa.[5] The time course of the reaction is shown in Figure 1(a). The reaction yield of acetate **2** reached approximately 50% at 5 h.

2.3. Effect of pressure on the reaction using Novozym at 55°C

The effect of pressure on the enantioselective acetylation of (*RS*)-**1** with vinyl acetate in $scCO_2$ by Novozym was investigated at 55°C changing the reaction time from 2 to 4 h.[5] Below 8 MPa, the reaction hardly proceeded, and the reaction above 19 MPa was not examined. As shown in Figure 1(b), the E value

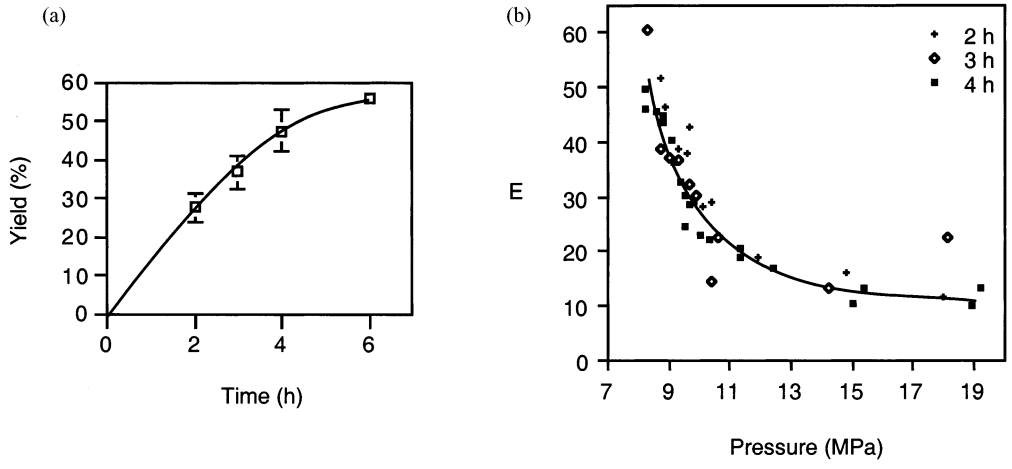

Figure 1. Acetylation of (*RS*)-**1** in supercritical carbon dioxide catalyzed by lipase Novozym at 55°C (a) time course at 10 MPa, (b) effect of pressure on enantioselectivity.

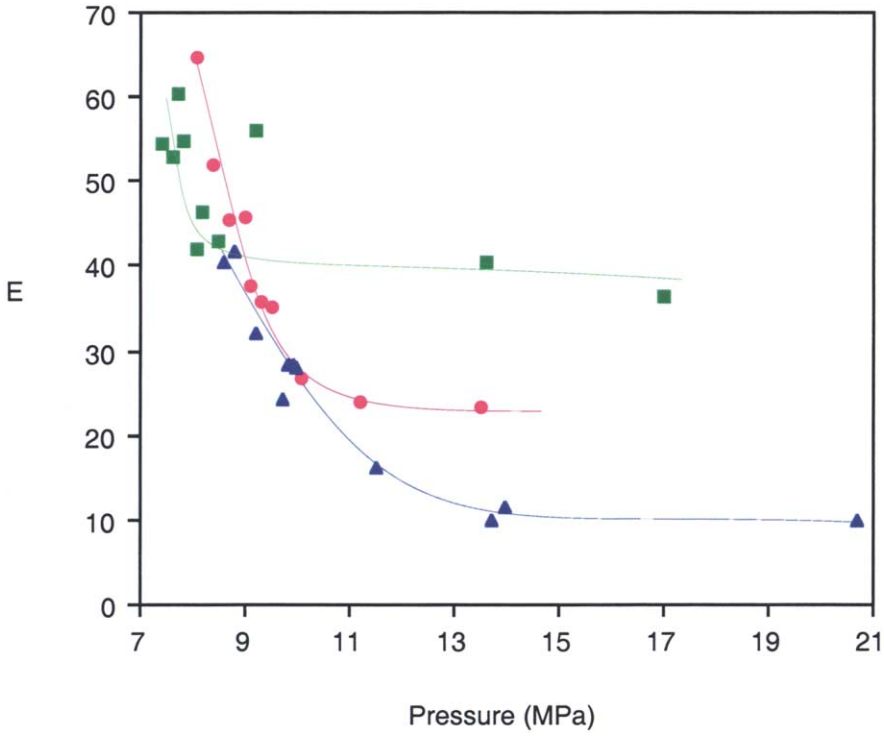

Figure 2. Effect of pressure on enantioselectivity of acetylation of (*RS*)-**1** in supercritical carbon dioxide catalyzed by lipase Novozym at 31°C (green), 40°C (magenta) and 60°C (blue).

changed continuously from 50 to 10 when the pressure was changed from 8 to 19 MPa, regardless of the reaction time.

The change in enantioselectivity of the reaction by the pressure is indeed noteworthy, although the reason is not clear at present. When the pressure of $scCO_2$ was changed, there was no significant change in the polarity evaluated as dielectric const. (at 50°C; 1.12 at 8 MPa and 1.29 at 11 MPa)[10] and log *P* (at 50°C; 1.4 at 8 MPa and 1.9 at 11 MPa).[11] It is not clear if this small change in polarity has a large effect on this reaction. On the other hand, the density of $scCO_2$ does change from 0.20 to 0.42 kg/L when the pressure is changed from 8 to 11 MPa at 55°C.[7] Ikushima explained the high enantioselectivity of lipase in a very limited pressure range at 304.1 K as resulting from the interaction between the CO_2 and enzyme molecules.[3] We also propose that the large change in density can significantly change the interaction of CO_2 and enzyme, causing formation of carbamates from CO_2 and the free amine groups on the surface of the enzyme[2e], CO_2 adsorption on the enzyme as reported in other proteins[12] and/or CO_2 incorporation in the substrate-binding pocket of the enzyme as reported in the incorporation of organic molecule in enzymes.[13] These interactions may gradually change the conformation of the enzyme in response to pressure, resulting in a continuous change in enantioselectivity. This conformational change is reversible because treatment of the enzyme by $scCO_2$ does not alter reactivities or enantioselectivities.

2.4. Effect of temperature on the reaction using Novozym

Effect of pressure on the enantioselective acetylation of (*RS*)-**1** with vinyl acetate in $scCO_2$ by Novozym was investigated at different temperatures. The results of the experiments at 31, 40 and 60°C are shown in Figure 2. As in the case at 55°C, the (*S*)-enantiomer reacted faster than the (*R*)-enantiomer, affording (*S*)-**2** and the remaining (*R*)-**1**, and the *E* value changed continuously according to the pressure. The cause of this change is probably due to the change in density as in the case at 55°C. This explanation agrees with the following observations. At lower temperatures (31, 40°C), the *E* values changed rapidly from high to low values within a small range of pressure below 10 MPa. However, at higher temperatures (55°C, 60°C), the *E* values changed gradually within a larger range of pressure below 14 MPa. These changes correlate well with the change in density; at low temperatures, the density changes rapidly from 0.2 to 0.6 g/mL below 10 MPa but at high temperatures, it changes gradually over a large range of pressure, as shown in Figure 3.[7]

However, when *E* values of the same density (at different temperatures and different pressures) were compared, *E* values were affected by the temperature. The higher the temperature, the lower the enantioselectivity. The enantioselectivity is determined not only by the density but also by the temperature. In a reaction under ambient conditions, the enantioselectivity in the kinetic resolution is temperature-dependent and obeys the following modified Eyring equation.[9,14]

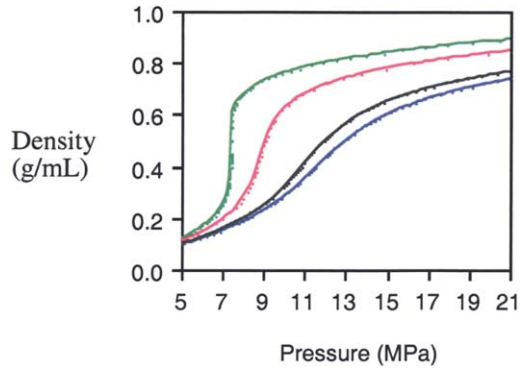

Figure 3. Density versus pressure of CO_2 at 31°C (green), 40°C (magenta) 55°C (black) and 60°C (blue).[7]

$$\ln E = -(\Delta\Delta H^{\neq}/R)(1/T)+(\Delta\Delta S^{\neq}/R)$$

By enzymatic reactions performed at temperatures ranging from 30 to −50°C, Sakai et al. showed the first experimental evidence supporting the theory of the effect of temperature on stereochemistry.[14] However, to the best of our knowledge, there are no experimental examples for the $scCO_2$ reaction. Here, we examined whether the theory is applicable to the reaction in $scCO_2$. At the density of 0.75 g/mL (31°C at 9.5 MPa, 35°C at 11.2 MPa, 40°C at 13.2 MPa, 45°C at 15.3 MPa, 50°C at 17.5 MPa, 55°C at 19.6 MPa, and 60°C at 21.8 MPa), ln E was plotted against $1/T$. As shown in Figure 4, the Eyring plot was found to be linear, which indicates the conformational stability of the transition state at a temperature range from 31°C to 60°C in $scCO_2$ at 0.75 g/mL. This is the first example showing that Eyring's theory can be applied to the biocatalytic reaction in $scCO_2$.

The observation in the variation of the enantioselectivity by changing the temperature at the same density, i.e. at the same dielectric constant,[10] is in contrast to the case for the transglycosylation by a lipid-coated β-D-galactosidase[2a] or the enantioselective esterification by a lipid-coated lipase[2b] in supercritical fluoroform by Mori et al. In these reports, reactivities and selectivities were controlled by the dielectric constant and not solely by

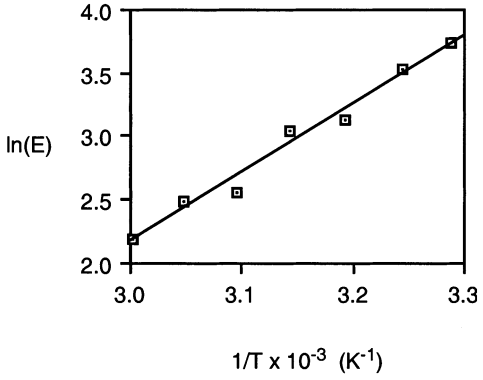

Figure 4. Effect of temperature on enantioselectivity of acetylation of (*RS*)-**1** in supercritical carbon dioxide catalyzed by lipase Novozym at 0.75 g/mL.

temperature or pressure. This contrasting result is probably due to the difference between fluoroform and CO_2 in the magnitude of the change in dielectric constants caused by the manipulation of pressure and temperature. In our case, both the density and the temperature controlled the reaction, but in their case, the effect of temperature on the reactions were probably negligible compared to the effect of the dielectric constant.

3. Conclusions

The enantioselectivity of an environmentally benign reaction was examined, and the continuous change in enantioselectivity without changing the molecular structure of the solvent, which is not possible by simply changing the organic solvent, was observed using $scCO_2$. The enantioselectivity of the reaction depends not only on the density but also on the temperature, and the Eyring plot was found to be linear. So we believe this work to be helpful to further developments in studying the origin of enantioselectivity and synthesizing useful compounds while keeping harmony with the natural environment.

4. Experimental

4.1. General

Lipase LPL was kindly supplied by Toyobo, the lipases AY, AH, PS-D and PS-C were kindly supplied by Amano Enzymes Inc., and the lipases Lipozyme and Novozym were kindly supplied by Novozymes. Chemicals were purchased from Nacalai Tesque, Inc., Wako Pure Chemical Industries, Ltd, and Aldrich Chemical Co and used without further purification unless otherwise indicated. Vinyl acetate was distilled and dried over MS-4A before use. Gas chromatographic analyses were performed using chiral GC-columns (Chrompack, Chirasil-DEX CB: 25 m; He 2 mL/min) equipped on the Shimadzu GC-14B with C-R7A plus.

4.2. Experimental apparatus for $scCO_2$ reactions

The apparatus consists of a CO_2 gas cylinder, cooler (−10°C), pump (Jasco PU-1580 pump), manometer (Taiatsu Techno, Co., Osaka, 15 MP or 25 MPa), stainless steel pressure-resistant vessel (Taiatsu Techno, Co., Osaka, TVS-N2 type, 10 mL), stop valve (Swagelok, SS3NBS4), oven and magnetic stir (Koike, HE-16GA).

4.3. Enantioselective acetylation of (*RS*)-1 in $scCO_2$

The lipase (Novozym, 25 mg), the racemic alcohol (*RS*)-**1** (2.5 mg, 0.012 mmol), vinyl acetate (0.025 mL, 0.27 mmol), and a magnetic stirrer bar were charged in the vessel (the chemicals were placed in a glass tube to prevent them from contacting the biocatalyst before supercritical conditions were achieved). The vessel was then warmed to 55°C, and CO_2, preheated to 55°C, was introduced at 9.3 MPa. The mixture was then stirred at

55°C for 3 h and the $scCO_2$ was liquefied at −10°C; subsequently, the gas pressure was released. The resulting residue was dissolved in ether, and the mixture put on Extrelut and quickly eluted with ether. The ee values of both the remaining alcohol **1** and the producing acetate **2** were measured with a chiral GC-column (130°C for 6 min followed by 10°C/min to 180°C) to determine the yields and E values. The absolute configurations were determined by comparing the GC retention times with those of authentic samples.[15] The experiment was conducted using other lipases and at different reaction times, temperatures or pressures.

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